AOAC Official Method 2001.03
Dietary Fiber in Foods Containing Resistant Maltodextrin
High MW RMD by 985.29 (IDF and SDF) and Low MW RMD by Liquid Chromatography
First Action 2001
Final Action 2005

[Applicable to resistant maltodextrin (RMD) and to foods containing RMD listed in Table **2001.03** at ≥1.4% RMD.]

A. Principle

This method determines total dietary fiber (TDF) value of processed foods containing insoluble dietary fiber (IDF) and high molecular weight soluble dietary fiber (HMWSDF), which are precipitated in ethanol and low molecular weight resistant maltodextrin (LMWRMD), which is soluble in ethanol. This method defines dietary fiber (DF) as consisting of nondigestible carbohydrates having a degree of polymerization with 3 sugar moieties (DP3) or higher after enzymatic hydrolysis. All the starches contained in food are converted to glucose after this enzymatic hydrolysis. This method to determine TDF content in processed foods containing RMD is a combination of **985.29** (*see* 45.4.07) for DF and a LC method for LMWRMD. A food is first analyzed for the total quantity of IDF and HMWSDF, precipitated in ethanol, according to **985.29** (*see* 45.4.07). Then an LC determination is conducted on the desalted filtrate to obtain the quantity of LMWRMD not precipitated in the 78% alcohol preparation. These 2 values [(IDF + HMWSDF) + LMWRMD] are summed to obtain the TDF value in the food.

B. Apparatus

(**a**) *Balance*.—Analytical, weighing to 0.1 mg.

(**b**) *Beakers*.—Tall-form, 500 mL.

(**c**) *Water baths*.—To maintain a temperature of 95°–100°C and 60°C with ability to shake the containers.

(**d**) *Filtering crucibles*.—Coarse, ASTM, 40–60 μm pore size, Pyrex, 50 mL.

(**e**) *Glass or plastic columns*.—To hold ion exchange resins, 75 cm × 15 mm id; a shorter (40–75 cm × 15 mm id) column can also be used.

(**f**) *Liquid chromatograph*.—With oven to maintain a column temperature of 80°C and a 20 μL injection loop. Column operating conditions are: Temperature, 80°C; mobile phase, distilled water, **C**(**d**); flow rate, 0.5 mL/min.

(**g**) *Guard column (or precolumn)*.—TSK® guard column PWXL, 6.0 mm id × 4 cm (Tosoh Corp., distributed by TosoHaas, Montgomeryville, PA, USA; www.tosohbiosep.com) or equivalent.

(**h**) *LC columns*.—Two LC columns connected in series, TSK-GEL® G2500PWXL, 7.8 mm id × 30 cm (Tosoh Corp.), or equivalent.

(**i**) *Detector*.—Refractive index (RI); maintained at 40°C.

(**j**) *Data integrator or computer*.—For peak area measurement.

(**k**) *Filters for disposable syringe*.—0.2 μm membrane, 13 mm.

(**l**) *Filters for water*.—0.2 μm, 47 mm.

(**m**) *Filter apparatus*.—To hold 47 mm, 0.2 μm filter, (**l**); to filter larger volumes of water, **C**(**d**).

(**n**) *Glass rods*.—With fire-polished ends, ca 20 cm long.

(**o**) *Syringes*.—10 mL, plastic disposable.

(**p**) *Pasteur pipet*

(**q**) *Volumetric pipet*.—10 mL.

(**r**) *Volumetric flasks*.—10, 50, 250, and 1000 mL.

(**s**) *Top loading balance*.—4000 g capacity.

(**t**) *Tubing*.—PVC, 2.79 mm id (for ion exchange columns).

(**u**) *Glass LC syringe*.—50 μL.

(**v**) *Teflon scraping rod*.—Use in place of glass stirring rod to scrape precipitate from tall-form beaker.

(**w**) *Rotary evaporator*.—R-3000VW "Student" (Büchi, Switzerland; www.buchi.com) or equivalent.

C. Reagents

(**a**) *Ethanol*.—95%. Technical grade, used at 60°C.

(**b**) *Ethanol*.—78%. Place 207 mL water in 1 L volumetric flask and dilute to volume with 95% ethanol, (**a**).

(**c**) *Acetone*.—Reagent grade.

(**d**) *Distilled water*.

(**e**) *Sodium phosphate dibasic*.

(**f**) *Sodium phosphate monobasic*.

(**g**) *Phosphate buffer*.—0.08M, pH 6.0. Dissolve 1.400 g Na_2HPO_4 (or 1.753 g dihydrate) and 9.68 g $NaH_2PO_4 \cdot H_2O$ (or 10.94 g dihydrate) in ca 700 mL water, (**d**). Dilute to 1 L with water, (**d**), and verify pH with pH meter.

(**h**) *Heat stable α-amylase solution (Termamyl)*.—No. 120L (activity: 12 units/mg protein; Novo Laboratories, Inc., 59 Danbury Rd, Wilton, CT 06897, USA), or equivalent (should not contain glycerol).

(**i**) *LC retention time standard*.—Standard source of the distribution of oligosaccharides (DP ≥3) in the LMWRMD fraction of RMD, corn syrup solids (DE 25; Matsutani Chemical Industry Co., Ltd., Itami City, Hyogo, Japan; www.matsutani.com), analyzed by LC (Figure **2001.03A**) as in **D**.

(**j**) *Protease*.—No. P-3910 or P-5380 (activity: 7–15 units/mg protein; Sigma Chemical Co., St. Louis, MO, USA), or equivalent (should not contain glycerol). Prepare protease stock solution just before use by adding 100 mg protease enzyme to a 10 mL volumetric flask and bringing to volume with water, (**d**), (amount is sufficient for ≥9 test portions in duplicate).

(**k**) *Amyloglucosidase*.—No. A-9913 (activity: 400 units/mg protein; Sigma Chemical Co.), or equivalent (should not contain glycerol).

(**l**) *Celite*.—No. C-8656 (Sigma Chemical Co.) or No. C-211, acid washed (Fisher Scientific Co., Fair Lawn, NJ, USA), or equivalent.

(**m**) *Mixed-bed ion exchange resins for each test portion*.—(*1*) *m-1*.—25 g Amberlite IRA-67 (OH-type; Organo Corp., Tokyo, Japan, www.bioscorpio.com/organo_corp.htm), or equivalent.

(*2*) *m-2*.—25 g Amberlite 200 CT(HG)H (H-type; Organo Corp.), or equivalent, are mixed and packed in column for analysis of each test portion. The converted resin should satisfy the following specifications: (*a*) Total ion exchange capacity: 1.74 meq/mL (min); (*b*) Effective ion exchange capacity (R-H exchange capacity): 1.6 meq/mL (min); (*c*) pH: 4–7. Before mixing and packing the 2 resins into a column, wash each resin with H_2O to obtain a pH value of 7–8.8 for *m-1* and 4–7 for *m-2*. If Amberlite 200CT(HG)H cannot be obtained, Amberlite 200 (Na-type; Sigma Chemical Co.) or Amberlite 200CT (Organo Corp.) can be used by converting it to "H-type" by the following procedure:

Fill column (100 cm × 40 mm id), **B**(**e**), with 600 mL (500 g) Amberlite 200 "Na-type" resin and determine approximate resin volume. Wash resin with 2 volumes of water, (**d**), at the rate of 60 mL/min. Pass 2 volumes of 10% HCl (1 + 3, w/w) through the resin at the rate of 60 mL/min. Remove HCl with 3 volumes of water, (**d**), passed through the resin at the rate of 60 mL/min. Add 3–6 vol-

© 2005 AOAC INTERNATIONAL

umes of additional water, (**d**), at the rate of 120 mL/min. The column is adequately washed of HCl when a pH value of 4–7 is obtained. (It takes 2–3 h to charge and rinse these resins.)

(**n**) *Sodium hydroxide.*—0.275M; reagent grade. Dissolve 11.0 g NaOH in ca 700 mL water, (**d**), in a 1 L volumetric flask. Dilute to volume with water, (**d**).

(**o**) *Hydrochloric acid.*—0.325M; reagent grade. Dilute stock solution of known titer, e.g., 325 mL 1M HCl, to 1 L with water.

(**p**) *Glycerol (LC standard).*—10 mg/mL. For stock solution: weigh 10 g glycerol >99.5% purity into a small beaker. Quantitatively transfer to 1 L volumetric flask with repeated washes with water, (**d**), and dilute to volume. It is important to measure and record the exact weight of the glycerol, weighing as close to 10 g as possible. Take purity and weight of glycerol into consideration when calculating concentration of final glycerol LC standard.

(**q**) *Glycerol (for dextrose–glycerol standard).*—100 mg/mL. Weigh 10 g high purity glycerol into a small beaker, transfer to a 100 mL volumetric flask with water, (**d**), and dilute to volume.

(**r**) *Ammonium sulfate.*—Reagent grade; standard to test micro-Kjeldahl procedure.

(**s**) *Dextrose.*—LC grade, high purity >99.5%.

(**t**) *Silver nitrate solution.*—0.1M. Dissolve 1.70 g $AgNO_3$ in ca 70 mL water, (**d**), in a 100 mL volumetric flask, and dilute to volume with water, (**d**).

D. Determination

(**a**) *Enzymatic hydrolysis and filtration.*—Weigh 1.0 g test portion (crushed, sieved to 10 mesh, fat extracted if >10% fat, and dried) into a 500 mL previously weighed tall-form beaker, **B(b)**. Prepare in duplicate with 2 blank digestion determinations. Disperse in 50 mL 0.08M phosphate buffer, **C(g)**, and sonicated to ensure complete hydration. Add 100 µL heat stable α-amylase, **C(h)**, and cover beaker with aluminum foil. Place beaker in shaker water bath and hold at 95°C for 30 min with shaking. Cool to room temperature, and adjust the pH of the solution to pH 7.5 ± 0.1 with 0.275M NaOH, **C(n)**. Add 0.5 mL protease solution, **C(j)**, and digest solution for 30 min at 60°C. Cool solution to room temperature (25°C), and adjust pH to 4.5 ± 0.2 with 0.325M HCl, **C(o)**. Add 0.3 mL amyloglucosidase, **C(k)**, and digest at 60°C for 30 min. Upon completion of the 3 enzyme digestion sequence, add 4 volumes of 95% ethanol, **C(a)**, by weight, previously heated to 60°C. Use the top loading balance to weigh beaker with digestion mixture when adding ethanol (obtain tare weight of beaker before adding test portion). Assay the 2 blank digestions (i.e., 2 beakers and 2 crucibles) in an identical manner.

Let solutions stand overnight to form a precipitate. Filter by suction, using a water aspirator or vacuum pump, through 1.0 g Celite layered on a Pyrex glass crucible filter that previously has been dried to constant weight. Wash the 500 mL tall-form beaker and the residue 3 times with 20 mL 78% ethanol, **C(b)**, 2 times with 10 mL 95% ethanol, **C(a)**, and 2 times with 10 mL acetone, **C(c)**.

Quantitatively transfer filtrate and washings to a 1 L round bottom flask. Dry residue in an air oven at 105°C overnight and record weight. This residue weight, minus the protein, ash, and blank residue weights represents the weight of the dietary fiber (IDF + HMWSDF) recovered by the AOAC method.

(**b**) *Filtrate recovery, desalting, and LC analysis.*—Evaporate with a rotary evaporator to near dryness. Dissolve the residue with a minimum amount of water, **C(d)**, and transfer quantitatively to a 50 mL volumetric flask. Add 10 mL of 10 mg/mL glycerol LC standard and dilute to volume with water, **C(d)**. Transfer contents of the 50 mL volumetric flask to a column (75 cm × 15 mm id) containing 25 g each, thoroughly mixed, of Amberlite IRA-67 (*m-1*) and Amberlite 200CT(HG)H (*m-2*) prepared just before use. Wash extract through the column with 250 mL water, **C(d)**, at the rate of 0.8 mL/min.

Collect 250 mL eluant from the ion exchange column and quantitatively transfer into a 500 mL round bottom flask. Evaporate to near dryness and quantitatively transfer to a 10 mL volumetric flask and dilute to volume with water, **C(d)**. Transfer the contents of the 10 mL volumetric flask to a 10 mL disposable syringe, **B(o)**, and filter through a 0.2 µm filter, **B(k)**. Use a 50 µL LC glass syringe, **B(u)**, to fill the 20 µL injection loop on the LC, **B(f)**.

(**c**) *Determining the response factor for dextrose; dextrose is equivalent to RMD in LC response.*—Each chromatogram must be evaluated or standardized for the RI response of RMD. This is accomplished using glycerol standard, **C(q)**. The peak areas, representing concentration, obtained by LC analysis of equal amounts of RMD and dextrose are equivalent. Glycerol is used as the internal standard but its peak area compared to the peak area for an equal amount of dextrose or RMD is not equivalent. A glycerol standard curve is therefore prepared to obtain a "response factor" to calculate the exact amount of RMD in a chromatogram of each test portion.

Prepare 3 solutions in individual 100 mL volumetric flasks containing the same amount of glycerol and 3 levels of dextrose. It is important to know and use the reported content (i.e., >99.5% purity) of both glycerol and dextrose standards as reported by suppliers. Accurately weigh 0.5, 1.0, and 2.0 g dextrose into 3 separate 100 mL volumetric flasks, respectively. To each flask add 10 mL of the 100 mg/mL glycerol standard, **C(q)**. Dilute each flask to volume with water, **C(d)**. These 3 flasks represent the standard solutions to

Table 2001.03. Interlaboratory results for the determination of total dietary fiber in selected foods containing resistant maltodextrin by enzymatic-gravimetric method and liquid chromatography

Food	$\bar{x}$, %	No. labs[a(b)]	s_r	RSD_r, %	s_R	RSD_R, %
Resistant maltodextrin	95.36	8(0)	1.63	1.71	2.37	2.48
Hard candy	37.99	7(1)	0.58	1.53	0.68	1.79
Chicken and vegetable soup	25.41	8(0)	0.74	2.89	1.18	4.65
Grapefruit juice	1.38	8(0)	0.02	1.33	0.04	3.20
White bread	9.60	8(0)	0.33	3.41	0.64	6.66
Strawberry Jell-O	9.91	8(0)	0.60	6.10	0.93	9.39

a(b) a = Number of laboratories retained after eliminating outliers; b = number of laboratories removed as outliers.

© 2005 AOAC INTERNATIONAL

calculate the "response factor" for dextrose that is used to determine the amount of RMD as displayed in LC chromatograms.

Use a 50 μL LC syringe, **B(u)**, to fill the 20 μL injection loop for each standard glycerol–dextrose solution. Obtain the values for the peak areas of dextrose and glycerol from the 3 chromatograms. The reciprocal of the slope obtained by comparing the ratio of peak area of dextrose/peak area of glycerol (y-axis) to the ratio of the weight of dextrose/weight of glycerol (x-axis) is the "response factor." The average "response factor" among laboratories is 0.82, varying slightly in each laboratory.

$$\text{Response factor} = 1 / (\text{PA-dex} / \text{PA-gly}) \cdot (\text{Wt-gly} / \text{Wt-dex})$$

where PA-dex = peak area dextrose; PA-gly = peak area glycerol; Wt-dex = weight of dextrose in standard; Wt-gly = weight of glycerol in standard.

A flow diagram for a combined enzymatic-gravimetric method and LC determination is shown in Figure **2001.03B**.

E. Calculations

All values used in calculations are in mg, except for percent (%) values.

Assay each test portion in duplicate, resulting in 2 test portion weight values, test portion weight and test portion weight' (prime); 2 crucibles for each blank and test portion, blank and blank' (prime); and test portion and test portion' (prime).

(**a**) *Calculate average % (IDF + HMWSDF) as follows.*—(*1*) Blank ash (Ab) = (ash + Celite + blank crucible) – (Celite + blank crucible).

(*2*) Blank residue weight (BRW) = ((BR + BR')/2) – (Pb + Ab) where Pb = blank protein, determined by micro-Kjeldahl procedure; BR = weight of first blank crucible with residue; BR' = weight of second blank crucible with residue; Ab = weight of blank ash from step (**a**)(*1*).

(*3*) Test portion residue weight (SR) = (residue + Celite + test portion crucible) – (Celite + test portion crucible). Duplicate test

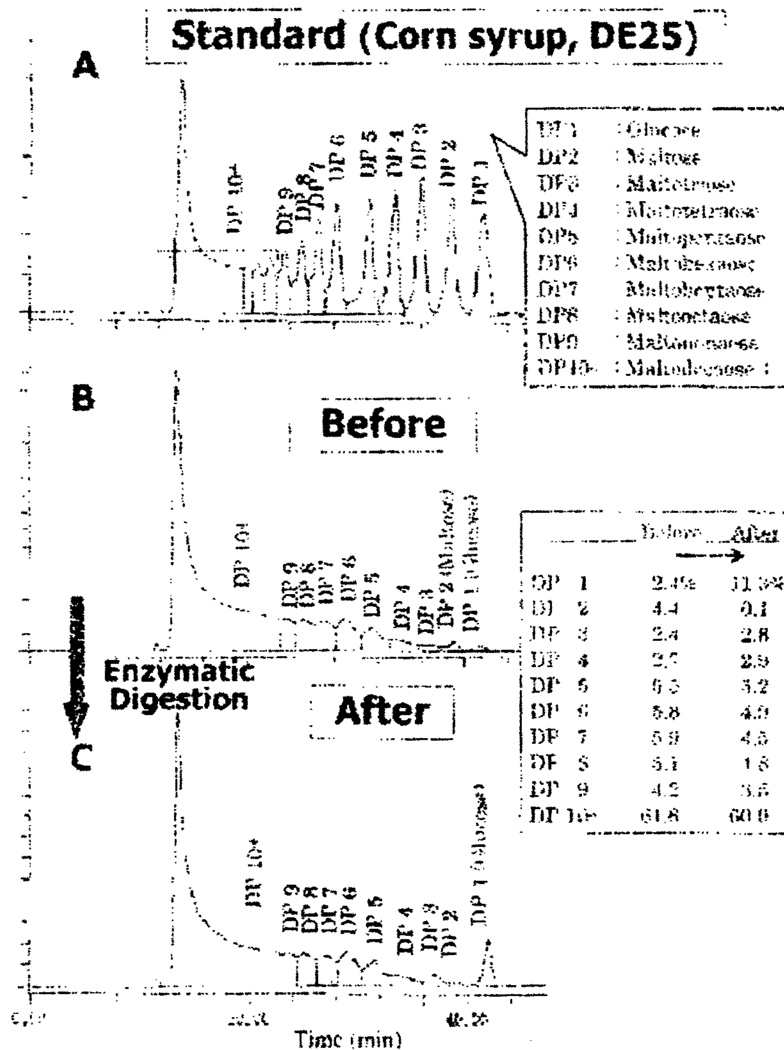


Figure 2001.03A. Liquid chromatogram of DE-25 corn syrup (A), resistant maltodextrin before treatment with hydrolytic enzymes (B), and resistant maltodextrin after treatment with hydrolytic enzymes (C).

© 2005 AOAC INTERNATIONAL

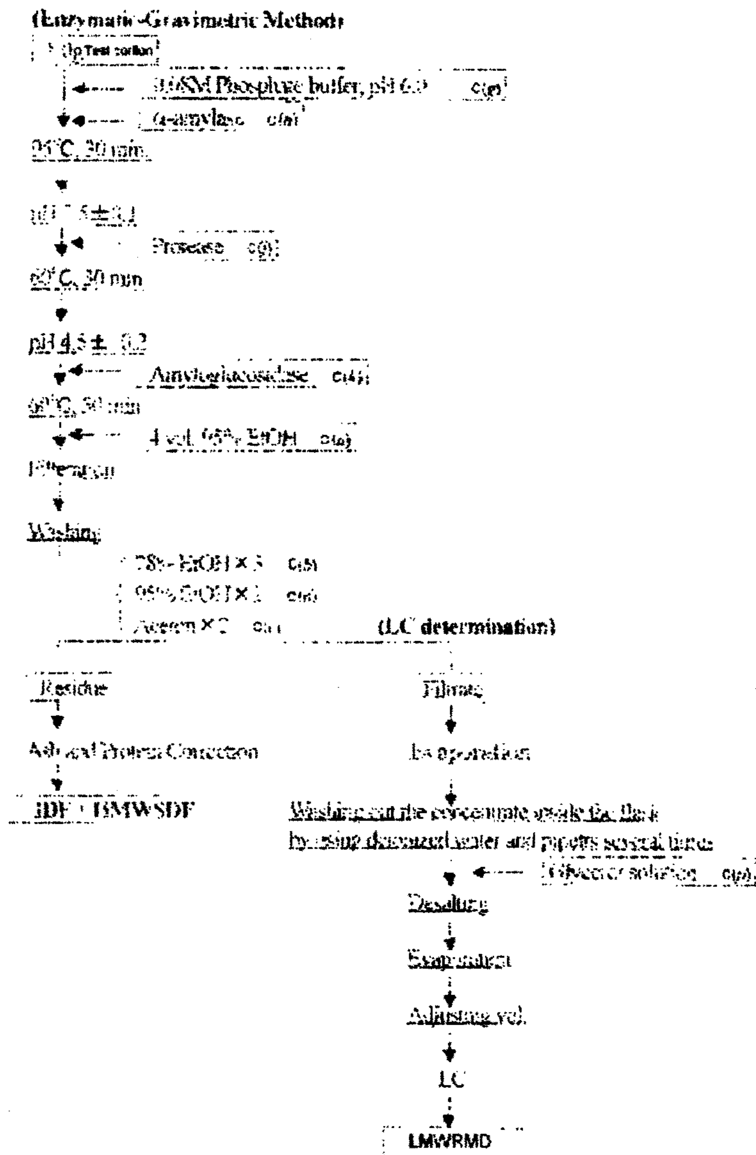


Figure 2001.03B. Flow diagram for a combined enzymatic-gravimetric method and LC determination.

portion residue weight (SR′) = (residue′ + Celite′ + test portion crucible′) – (Celite′ + test portion crucible′).

(*4*) Test portion ash weight (As) = (ash + Celite + crucible) – (Celite + crucible).

(*5*) Final test portion residue weight (FSR) = SR – Ps – As – BRW = FSR where Ps = protein, determined by micro-Kjeldahl procedure; SR = final test portion residue weight from step (**a**)(*3*); As = test portion ash weight from step (**a**)(*4*); BRW = blank residue weight from step (**a**)(*2*). Repeat this calculation for FSR′ using SR′ – Ps – As – BRW (using values from duplicate test portion weights).

(*6*) Percent final test portion residue weight (% FSR) = (FSR / SW) × 100 = % FSR where FSR = final test portion residue weight from step (**a**)(*5*); SW = test portion weight. Repeat this calculation for % FSR′ using FSR′ and SW′.

(*7*) %(IDF + HMWSDF) = average % FSR = (% FSR + % FSR′) / 2 where % FSR = percent final test portion residue weight; % FSR′ = percent final duplicate test portion residue weight.

(**b**) *Calculate average % LMWRMD as follows.*—(*1*) LMWRMD = (peak area of LMWRMD / peak area of glycerol) × (glycerol standard, mg × response factor).

(*2*) % LMWRMD = (LMWRMD / SW) × 100 where LMWRMD = weight of LMWRMD from step (**b**)(*1*); SW = test portion weight. Repeat calculations for % LMWRMD′ using LMWRMD′ and SW′.

(*3*) % ALMWRMD = average % LMWRMD = (%LMWRMD + % LMWRMD′) / 2 where % LMWRMD = percent LMWRMD for test

© 2005 AOAC INTERNATIONAL

portion from step (**b**)(*2*); % LMWRMD′ = percent LMWRMD for duplicate test portion from step (**b**)(*2*).

(**c**) *Calculate average % total dietary fiber (TDF) as follows.*—Percent (%) TDF = % (IDF + HMWSDF) + % ALMWRMD where % (IDF + HMWSDF) = average percent IDF + HMWSDF from step (**a**)(*7*); % ALMWRMD = average percent LMWRMD from step (**b**)(*3*).

F. Resistant Maltodextrin

The commercially available U.S. GRAS status RMD is a source of dietary fiber. Resistant maltodextrin is certified as an approved dietary fiber ingredient for the Program for Foods for Specific Health Use (FOSHU) in Japan. Dietary fiber supplements prepared simply by packaging RMD (or agglomerated RMD) in sachet forms and labeled as RMD have been on the market. Fibersol®-2, RMD, is manufactured and was supplied by Matsutani Chemical Industry Co., Ltd. (Itami City, Hyogo, Japan). The moisture content of the product is 2.7% and DE is 10.5. The RMD is produced by the pyrolysis and subsequent enzyme treatment of corn starch. It is an aggregate of glucose polymers with the MW distribution of 180 (DP-1) to >10 000 (DP-62) daltons, but the average MW is 2000 daltons. It contains α 1–4 and α 1–6 glucosidic bonds, which originate from starch and 1–2 and 1–3 glucosidic bonds that are created by transglucosidation during pyrolysis.

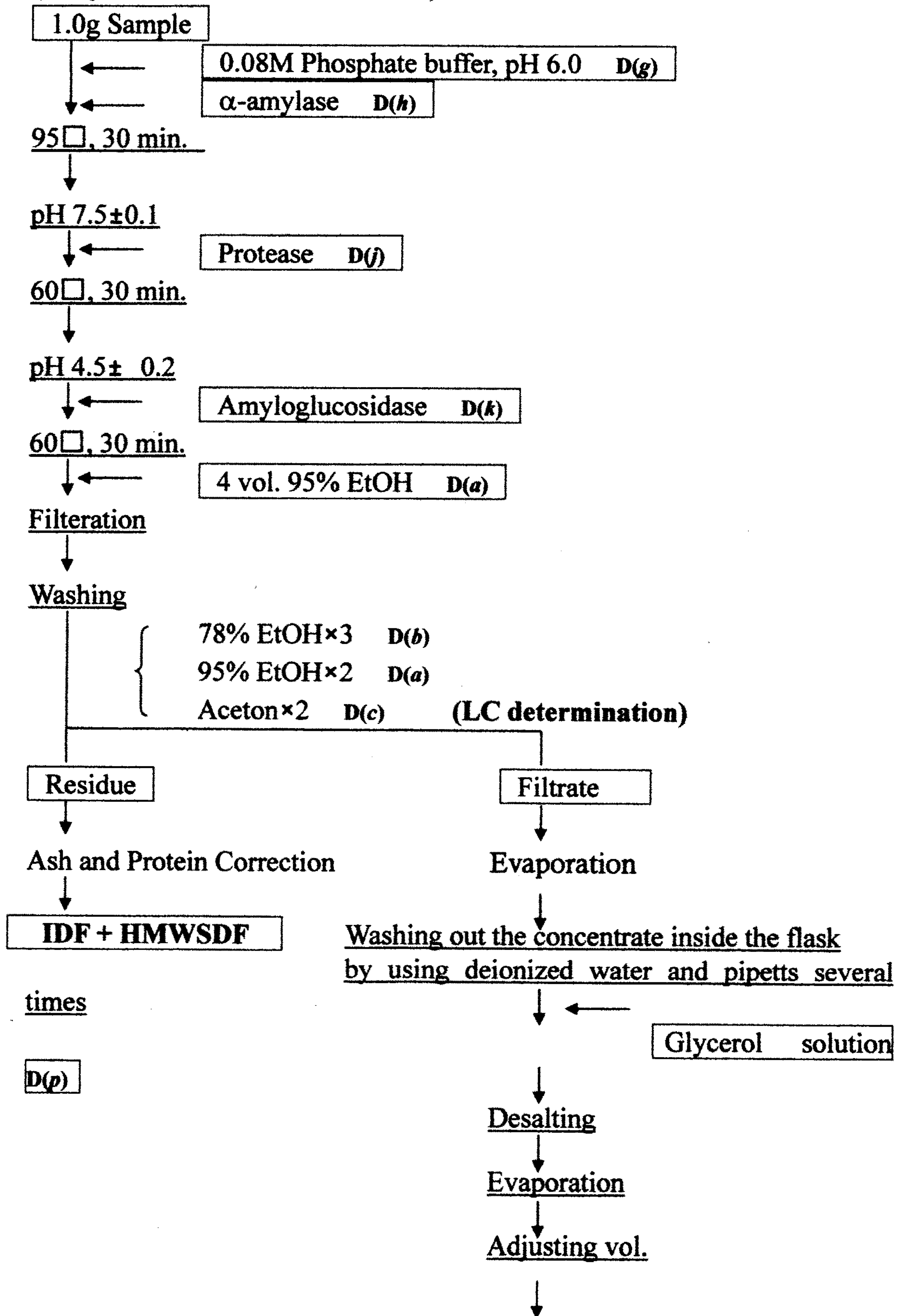
Internal utilization of RMD by in vitro and in vivo tests show that <10% is digested and absorbed in the small intestine. Approximately 50% of the products are fermented in the large intestine and ca 40% of the products are excreted into the feces. In order to distinguish this substance from conventional maltodextrin (digestible), the term "resistant" is added and used to describe this compound.

The sugar, oligosaccharide, and polysaccharide composition of the LMWRMD fraction of the RMD has been determined before and after hydrolytic enzyme treatments and is shown in Figure **2001.03A**. The distribution of these oligosaccharides is not significantly changed when RMD is treated with hydrolytic enzymes. To assess the oligosaccharide moieties and their distribution in the LMWRMD of RMD, corn syrup solids were used as a standard source of these oligosaccharides (Figure **2001.03A**). The nondigestible portions of RMD consists of DP units of 3 (DP-3) and above (Figure **2001.03A**). These nondigestible oligosaccharides and polysaccharides constitute >90% of RMD. Approximately 60% of RMD consist of polymers having >10 DP.

References: *J. AOAC Int.* **83**, 1013(2000); **85**, 435(2002).

© 2005 AOAC INTERNATIONAL

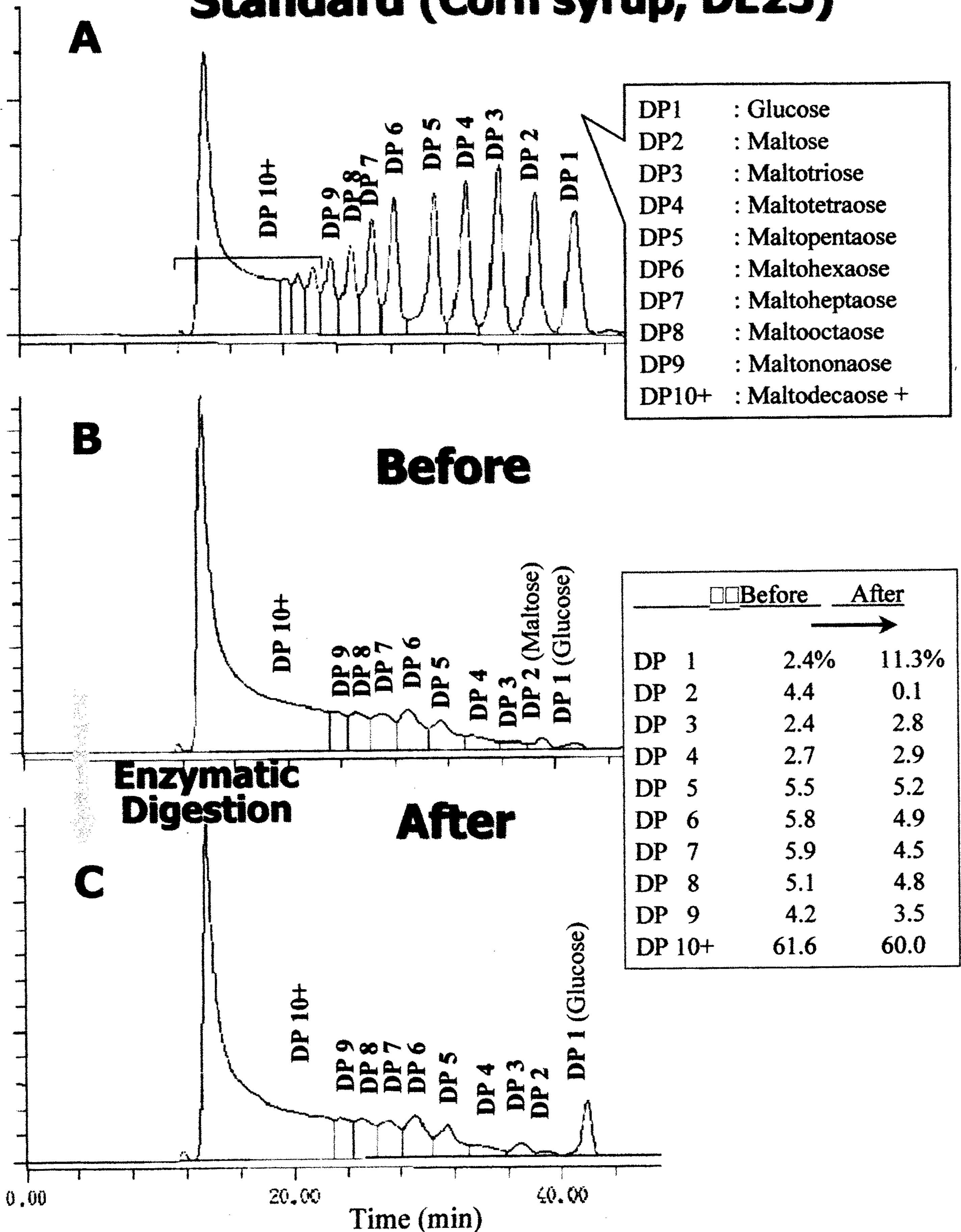
(Enzymatic-Gravimetric Method)



LC

LMWRMD

Figure 2001.03B: Flow diagram for a combined enzymatic-gravimetric method and LC determination.



	Before	After
DP 1	2.4%	11.3%
DP 2	4.4	0.1
DP 3	2.4	2.8
DP 4	2.7	2.9
DP 5	5.5	5.2
DP 6	5.8	4.9
DP 7	5.9	4.5
DP 8	5.1	4.8
DP 9	4.2	3.5
DP 10+	61.6	60.0

Figure 2001.03A: LC chromatogram of DE-25 corn syrup (A), resistant maltodextrin before treatment with hydrolytic enzymes (B) and, resistant maltodextrin after treatment with hydrolytic enzymes (C)